128 MULTIOMIC SPATIAL INTERROGATION OF TUMOR-INFILTRATED IMMUNE CELLS USING THE RNASCOPE™ CO-DETECTION ASSAY WITH VIVID DYES

[1]Anushka Dikshit*, [2]Sayantani Basak, [1]Emerald Doolittle. [1]*Advanced Cell Diagnostics, a Bio-Techne, Newark, CA, USA*; [2]*Bio-Techne, Newark, CA, USA*

Background Interrogating complex tumor microenvironment requires a multi-omics approach that can provide high level of sensitivity and specificity. Identifying immune cell subsets within the tumor can be vital for predicting response and determining therapeutic efficacy. Detecting target immune cell markers using immunohistochemistry/Immunofluorescence (IHC/IF) and visualizing cytokine expression with *in situ* hybridization (ISH) can provide comprehensive information about the activation states of immune cells. Here, we demonstrate a newly developed integrated ISH and IHC/IF workflow compatible with manual and automated platforms that can substantially improve RNA-protein co-detection.

Methods We demonstrate the use of our RNA-Protein Co-detection assay in combination with the automated and manual RNAscope Multiplex Fluorescent v2 assay and the RNAscope Chromogenic Duplex assay. The RNAscope Multiplex Fluorescent v2 assay was also combined with the new TSA Vivid dyes for detection of cytokines and immune cells. The co-detection assays enabled detection of T cell markers, macrophage markers and checkpoint markers in the tumor microenvironment by using a tumor microarray.

Results We identified CD4+ helper T cells and CD8+ cytotoxic T lymphocytes using CD3, CD8 and CD4 antibodies. Additionally, we determine the activation states of CD8+ T cells by visualizing *IFNG, GZMB and IL-2* RNA expression. We were also able to identify macrophages detected by CD68 protein expression, *CD163* and *ITGAM* RNA expression. We could also delineate tumor-stroma border in the samples by using the Pan-CK probe which distinctly marks the tumor cells and visualize the expression of immunoregulatory receptors PD-L1 and *CTLA4* in the tumor cells. The new TSA Vivid dyes demonstrated much brighter signals when compared to than other TSA-based dyes enabling robust detection of immune cell and low-abundance cytokine markers.

Conclusions The RNA-protein co-detection assay is enabled for multi-omic detection of target biomarkers with both chromogenic and fluorescent RNAscope assays. The new TSA vivid dyes improve the robustness of target detection using the RNAscope Multiplex V2 assay with co-detection by effectively boosting signal intensity. Overall, the new RNAscope RNA-protein co-detection workflow and reagents allow optimized simultaneous visualization of RNA and protein targets by enhancing the compatibility of antibodies with RNAscope.

http://dx.doi.org/10.1136/jitc-2022-SITC2022.0128